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Moina heilongjiangensis sp. nov. (Crustacea, Cladocera) from the northeast of China

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Abstract

A new species of *Moina* Baird, 1850 (Cladocera, Moinidae), is described from a brackish lake in the northeast of China based on morphology and molecular phylogenetics. The morphological description of *Moina heilongjiangensis* **sp. nov.** includes details of the thoracic limb structure, and we compare the new species with other members of the genus *Moina* based on key morphological characteristics. *Moina heilongjiangensis* **sp. nov.** is one of the few members of the genus with two eggs in the ephippium and lacking superficial hairs on the body. We also applied two genetic markers (COI and ITS-1) to assess its position in the genus *Moina*. Our molecular phylogenetic tree indicates that *M. heilongjiangensis* **sp. nov.** is close to *Moina affinis* Birge, 1893, based on mitochondrial sequences.

Key Words

Cladocera, *Moina heilongjiangensis* sp. nov., Moinidae, morphology, new species, phylogenetics, taxonomy

Introduction

The genus *Moina* Baird, 1850 (Cladocera, Moinidae) is a widespread taxon and a key component in aquatic ecosystems (Smirnov 1976; Van Damme and Dumont 2008), where it transfers energy from primary producers (i.e., phytoplankton) to higher trophic levels of consumers (i.e., fish) (Pace and Orcutt 1981; Lampert and Sommer 1999). *Moina* species have also been successfully used as model organisms in toxicological studies (Yi et al. 2010; Jia et al. 2018; Kwak et al. 2018). However, little attention has been paid to the taxonomy and molecular systematics of the genus, and new species have been described in recent years (Van Damme and Dumont 2008; Makino et al. 2019; Neretina et al. 2020; Deng et al. 2021; Neretina et al. 2024).

Since the revisions by Goulden (1968) and Smirnov (1976), more than twenty *Moina* species have been described based on morphology (i.e., Hudec 1987; Mirabdullayev 1998; Kotov et al. 2005; Van Damme and Dumont 2008; Dumont et al. 2013; Padhye and Dumont 2014). Some *Moina* species, such as *M. diksamensis* (Van Damme and Dumont 2008) and *M. siamensis* (Alonso et al. 2019), were described based on morphological characters only. The authors of those studies formulated a set of key morphological standards for species differentiation within the genus *Moina*. These included the thoracic limb structure and the number of eggs in the ephippium (Van Damme and Dumont 2008; Alonso et al. 2019; Neretina et al. 2020; Neretina et al. 2024).

In recent decades, molecular tools have been successfully applied in the molecular phylogenetics of *Moina*

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(Padhye and Dumont 2014; Bekker et al. 2016; Elías-Gutiérrez et al. 2019; Montoliu-Elena et al. 2019; Neretina and Kirdyasheva 2019; Deng et al. 2021). The first genetic study discovered that the "*Moina micrura*" from Europe and Australia were actually two different species (Petrusek et al. 2004). Some more recent studies applied DNA barcoding and detected cryptic species from the *M. brachiata* (Jurine, 1820) species complex in Hungary (Nédli et al. 2014) and northern Eurasia (Bekker et al. 2016). Most recently, an integrative taxonomic approach (combining morphology and molecular phylogeny) has been used for delimiting species in the genus *Moina* (Neretina et al. 2020). Other recent studies have successfully explored species diversity in *Moina* from Japan (Makino et al. 2019) and Nigeria (Deng et al. 2021) using this approach.

In China, nine *Moina* species have been recorded based on their morphology since the 1970s (Chiang and Du 1979): *M. brachiata* (Jurine, 1820); *M. macrocopa* (Straus, 1820); *M. affinis* Birge, 1893; *M. chankensis* Uéno, 1939; *M. weismanni* Ishikawa, 1896; *M. micrura* Kurz, 1874; *M. geei* Brehm, 1933; *M. rectirostris* (Leydig, 1860); and *M. irrasa* Brehm, 1937. This was reduced to eight species by Xiang et al. (2015): *M. brachiata*, *M. macrocopa*, *M. affinis*, *M. chankensis*, *M. weismanni*, *M. micrura*, *M. geei*, and *M. salina* Daday, 1888. *Moina micrura* s.lat. is the most widely distributed taxon in China, with records in all regions except for the Qinghai-Tibetan Plateau (Mashiko 1951; Chiang and Du 1979; Chen 1983; Chen and Hu 1988; Xiang et al. 2015; Ni et al. 2019). Our previous study detected four *Moina* species (*M. brachiata*, *M. macrocopa*, *M. micrura*, and *M. salina*) plus several new lineages in China supported by the mitochondrial (COI) and nuclear (ITS-1) genetic markers (Ni et al. 2019). Some of these lineages are likely to represent undescribed species if we apply more detailed morphological examination.

The present study describes a new species of *Moina* from northeast China, using morphological characters and molecular phylogeny.

Materials and methods

Sampling

Moina heilongjiangensis sp. nov. was collected from a brackish lake called Xidahai in Daqing, Heilongjiang Province, China (located at 46.03°N, 124.36°E) using a plankton net (mesh size 64 μm) hauled vertically through the water column at three different sites in the lake. Samples were pooled and preserved in 95% ethanol and deposited at the Preservation of Zooplankton Collection at Fudan University, Shanghai, China. Parthenogenetic clones of this new species were maintained in the laboratory in filtered COMBO medium (Kilham et al. 1998) at 20 °C under a 16:8 h light:dark cycle and fed with the unicellular alga *Ankistrodesmus falcatus* every two days. Salinity of the filtered COMBO medium was adjusted to 2.2 ppt, corresponding to the salinity of Xidahai during the sampling period.

Morphological examination

Moina specimens were identified based on morphological characters. Adult females belonging to the *M. heilongjiangensis* sp. nov. were selected from ethanol-preserved samples under a stereo microscope (SMZ-161, Motic). We added 8 μg methyl farnesoate (MF; Echelon Bioscience, Salt Lake City, UT, USA) to 100 ml COMBO medium to induce males in live cultures based on a previous protocol (Toyota et al. 2015). Ten adult female and six adult male individuals were examined for their morphology under a high-resolution optical microscope (ECLIPSE Ci-S, Nikon). Both female and male individuals were rehydrated through a series of ethanol solutions (75%, 50%, 25%), then dissected individually in 80% glycerol on a slide and covered with a coverslip (Andrade-Sossa et al. 2020). Line drawings of the specimens were based on microphotographs taken by a camera (DS-L3, Nikon) connected to the optical microscope using the software FCSnap.

DNA extraction and sequencing

DNA was extracted from individual specimens using the proteinase K method (Schwenk et al. 1998). Specifically, each specimen was added to 20 μL of H3 buffer (containing final concentrations of 10 mM Tris-HCl, 50 mM KCl, 0.005% Tween 20, and 0.005% NP-40) mixed with 0.1 mg/mL of proteinase K (MERCK, Germany). The mixed solution was then incubated in a 55 °C water bath with mild shaking for 16 h and finally held at 95 °C for 12 min to denature proteinase K. DNA samples were briefly centrifugated and stored at 4 °C for further analyses.

A 680-bp segment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene and an 810-bp segment of the nuclear ribosomal first internal transcribed spacer (ITS-1) were used as genetic markers. Two female individuals of *M. heilongjiangensis* were successfully sequenced at both loci. For COI, PCR amplification was conducted using the primer pair LCO1490 and HCO2918 (Folmer et al. 1994). The PCR was carried out in a total volume of 20 μL, consisting of 12 μL 2 × HieffTM PCR Master Mix (with dye), 3 μL ddH_2O, 1 μL 10 μM solution of each primer, and 3 μL DNA sample. The PCR amplification cycling conditions were as follows: incubation for 5 min at 94 °C, then 40 cycles of denaturation for 45 s at 94 °C, annealing for 45 s at 45 °C, and extension for 45 s at 72 °C, followed by a final extension for 7 min at 72 °C. Amplification of the ITS-1 was performed using primers 18SD and 5.8BR (Taylor et al. 2005). The PCR mixture was the same as for COI, except for the primers we used. The conditions for ITS-1 were as follows: incubation for 1 min at 94 °C, then 40 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 53 °C, and extension for 2 min at 72 °C, followed by a final extension for 7 min at 72 °C. Since the ITS-1 fragments contained multiple heterozygous sites, we used cloning to obtain

unambiguous chromatograms (Ni et al. 2019; Deng et al. 2021). The PCR products were recovered from an agarose gel, then we used the pGEM®-T Vector System I Kit (Promega, Beijing, China) to insert the PCR products into a pMD®19-T Vector plasmid (TaKaRa, Beijing, China) and transformed them into *E. coli* DH5α (TsingKe Biotech Co., Ltd, Beijing, China). The recombinant clones containing the insert fragment were verified by colony PCR (conditions as above), and then plasmids were extracted from bacteria using the HiPure Plasmid Micro Kit (Magen, Guangzhou, China) according to the manufacturer's protocol. Twenty clones were sequenced for each ITS-1 PCR product; only identical sequences/alleles obtained at least twice per individual were chosen for further analysis. All COI and ITS-1 PCR products were sequenced in the forward direction on an ABI PRISM 3730 DNA capillary sequencer, using the PCR primers as sequencing primers (Sangon Biotech Co., Ltd., Shanghai, China). All chromatograms were examined carefully and underwent manual correction for base-calling errors in MEGA X (Kumar et al. 2018). Sequences of *M. heilongjiangensis* have been submitted to GenBank under accession numbers COI: PV712684-PV712685 and ITS-1: PV708582-PV708585.

Genetic diversity and phylogeny

For COI, two different haplotypes of *M. heilongjiangensis* were identified using DNASP 6 (Librado and Rozas 2009) and subsequently aligned in MEGA X, along with all 121 published sequences of *Moina* species retrieved from GenBank (Suppl. material 1: table S1). Likewise, unique ITS-1 alleles were identified in DNASP 6 and aligned in MEGA X along with 30 published ITS-1 sequences of *Moina* retrieved from GenBank (Suppl. material 1: table S2). We assessed the potential loss of phylogenetic signal in COI sequences caused by substitution saturation using DAMBE 5 (Xia 2013). A COI phylogenetic tree using a Bayesian method was constructed in BEAST 1.8 (Bouckaert et al. 2014) with a tree recorded every 1,000 generations among 10,000,000 generations and a burn-in of 25%. Finally, 7,500 sampled trees were summarized using TreeAnnotator. COI sequences of all published *Moina* lineages were included in this tree. *Ceriodaphnia* Dana, 1853, a member of the Daphniidae, which forms a phylogenetic branch with Moinidae and Macrothricidae (Van Damme et al. 2022), was used as an outgroup. The best substitution model (General Time Reversible Model + Invariant Sites + Gamma, GTR + I + G) was determined through the corrected Akaike Information Criterion in jModeltest v. 2.1.7 (Darriba et al. 2012). Tracer v1.7 (Rambaut et al. 2018) was then applied to confirm that the analysis had computed adequate generations. Similarly, a Bayesian phylogenetic tree was constructed for the ITS-1 marker in BEAST using the General Time Reversible Model + Gamma (GTR + G; the best model found using jModeltest) substitution model.

Detection of new species and phylogeographic analyses

We used three independent species-delimitation methods, i.e., the general mixed Yule coalescent model (GMYC; Pons et al. 2006), the Poisson tree processes method (PTP, Zhang et al. 2013), and the automatic barcode-gap discovery method (ABGD, Puillandre et al. 2012), to detect new species/lineages of *Moina* for both COI and ITS-1 markers. The input was the phylogenetic tree generated using BEAST; see above. GMYC analysis was performed with a single threshold (Fujisawa and Barraclough 2013) on its webserver (https://species.h-its.org/gmyc/). The PTP method was performed on the bPTP webserver (http://species.h-its.org/ptp/), with 100,000 MCMC generations, thinning set to 100, burn-in at 25%, and performing a Bayesian search. ABGD analysis was conducted on the online server (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) with the default settings.

Data availability

All new sequences have been deposited in GenBank under accession numbers COI: PV712684-PV712685 and ITS-1: PV708582-PV708585.

Results

Taxonomy

Class Branchiopoda Latreille, 1817
Order Anomopoda Sars, 1865
Family Moinidae Goulden, 1968
Genus *Moina* Baird, 1850

***Moina heilongjiangensis* sp. nov.**

https://zoobank.org/AA5599AB-F212-408D-AF87-94A08D88705B

Figs 1–3

Etymology. The species is named after Heilongjiang, the province in China where the new species was first discovered.

Type locality. Xidahai, Daqing, Heilongjiang Province, China (a lake located at 46.03°N, 124.36°E). Xidahai is an inland brackish lake, with a length of 8.4 km and an average width of 3.1 km, covering an area of 26.5 km^2. The maximum depth is 3.5 m, with an average depth of 2.3 m. The annual mean temperature of the lake is 3.4 °C, with an average temperature of 23.1 °C in July (Wang and Dou 1998).

Holotype. An adult parthenogenetic female in 95% ethanol was deposited at the Preservation of Zooplankton Collection at Fudan University, Shanghai, China, 1.17 mm in length, from Xidahai, accession number: MHXDH2023090101.

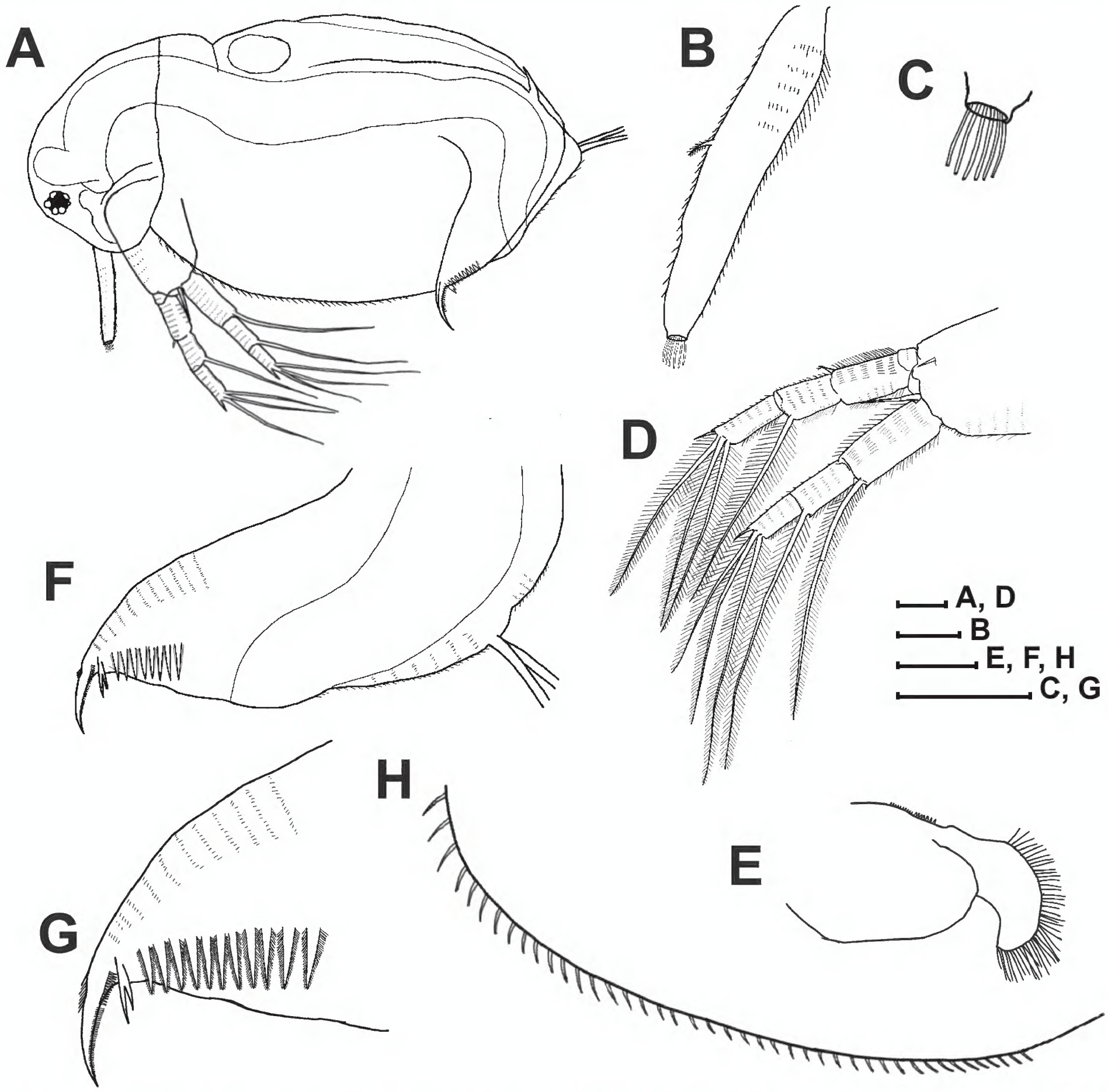

Figure 1. Morphology of *Moina heilongjiangensis*, parthenogenetic females from Xidahai, China. **A.** Adult parthenogenetic female, dorsal; **B, C.** Antenna I and its tip; **D.** Antenna II; **E.** Labrum, lateral view; **F.** Postabdomen, lateral view; **G.** Distal portion of postabdomen, lateral view; **H.** Valve from anterior portion to posterior portion. Scale bars: 0.1 mm.

Allotype. An adult male in 95% ethanol was deposited at the Preservation of Zooplankton Collection at Fudan University, China, 1.02 mm in length, from Xidahai, accession number: MHXDH2023090301.

Paratypes. All paratypes in 95% ethanol were deposited at the Preservation of Zooplankton Collection at Fudan University, China. Five adult parthenogenetic females, 1.14–1.35 mm in length, from Xidahai. Four ephippial females, 1.08–1.31 mm in length, from Xidahai, China. Four adult males, 1.00–1.18 mm in length, from Xidahai, China, accession numbers: adult parthenogenetic females: MHXDH2023090102-MHXDH2023090106, ephippial females: MHXDH2023090201-MHXDH2023090204, adult males: MHXDH2023090302-MHXDH2023090305.

Morphological description. Parthenogenetic female (Figs 1, 2). Body ovoid in lateral view, shape typical of genus. Relatively large, body height/length ratio about 0.46–0.63 for adults, depending on development of brood chamber. Dorsal margin of valve slightly higher than head level. Anterodorsal angle rounded, posteroventral angle broadly rounded (Fig. 1A). Ventral margin of valve rounded, with 40–45 marginal setae (Fig. 1A, H). Surface of head and valve lacking a cover of fine hair.

Head ovoid-round, lacking supra-ocular depression and dorsal head pore (Fig. 1A). Compound eye small, ocellus absent.

Labrum medium length, with fleshy main body, straight ventral margin (Fig. 1E). Short setulae on inner ventral portion and long setulae on labral plate.

Valve ovoid, without fine hairs (Fig. 1A). Setae relatively long on anterior portion of ventral margin, shorter on posterior portion (Fig. 1H).

Postabdomen typical of genus, conically narrowing distally (Fig. 1F, G). Postabdomen with curved ventral margin; preanal margin longer than anal, both with rows of short hairs; postanal region bears row of 7–11 large, triangular, feathered teeth.

Postabdominal claw relatively large, slightly curved, with pointed apex (Fig. 1F, G); dorsal side with two pectens, proximal ventral pecten well-developed, formed of short teeth; distal pecten formed of shorter fine setulae; ventral margin of claw with several denticles on root edge. Tooth near anterior portion of postabdominal claw bident, with branches unequal in length.

Antenna I long, almost straight (Fig. 1B, C). Antennular body with transverse long fine hairs and numerous minute denticles. One additional setule on antenna I. Antennular sensory seta relatively short, implanted at middle of antennular body. Six aesthetascs short, sub-equal in size (Fig. 1C).

Antenna II typical of moinids (Fig. 1D). Coxa with short setulated sensory setae and two elongated antennal branches. Basal segment robust, with numerous transverse rows of fine hairs; short spine on outer surface between exopods. One exopod with four cylindrical segments and one endopod with three cylindrical segments, covered by transverse rows of denticles and hairs. Antennal formula: setae 0-0-1-3/1-1-3; spines 0-1-0-1/0-0-1. Setae of both antennal branches long, with long, fine setulae. Spine on second exopod segment short, slightly shorter than apical exopod and endopod spines.

Limb I with elongated limb body (Fig. 2A). Endite 5 with one anterior seta (Fig. 2A, B: 1) covered by short setulae and two posterior setae (Fig. 2A: a-b). All posterior setae on limb I covered by short, dense setulae. Endite 4 with one anterior seta (Fig. 2A, B: 2) and a single posterior seta (Fig. 2A: c). Endite 3 without an anterior seta, but with two posterior setae (Fig. 2A: d, e). Endite 2 with three posterior setae (Fig. 2A: f–h). Two ejector hooks of different lengths (Fig. 2A: ejh).

Limb II with large ovoid epipodite (Fig. 2C: epp) and elongated exopodite bearing a single seta (Fig. 2C). Endite 5 with a slender soft seta and a reduced shorter soft seta (Fig. 2C: a, b); endite 4 with a single soft seta (Fig. 2C: c); endite 3 with a soft seta (Fig. 2C: d); endite 2 with a small soft seta implanted anteriorly on limb body and one slender posterior soft seta (Fig. 2C: e, f); gnathobase (endite 1) with four anterior setae (Fig. 2C: 1–4) and about 15 posterior setae (Fig. 2C: a–o). Seta 1 elongated, with relatively long setulae. Setae 2, 3, and 4 significantly shorter than seta 1, with short setulae located on basal corner of gnathobase (Fig. 2C).

Limb III with ovoid epipodite (Fig. 2D: epp). Exopodite (Fig. 2D: ext) almost rounded, with four distal and two lateral setae (Fig. 2D). Inner distal portion of limb with two endites: first endite bearing three posterior (Fig. 2D, E: 2–4) and ten anterior setae (Fig. 2D–F: a–j); second endite bearing two anterior setae (Fig. 2D, F: 1, 5) and six anterior (Fig. 2E, F: k–p) setae. Large lobe (gnathobase) on inner margin of limb, with numerous setae (Fig. 2D, F).

Limb IV with small ovoid epipodite (Fig. 2G: epp). Exopodite (Fig. 2G: ext) rounded, relatively large, as in limb III, with four distal and two lateral setae. Inner distal portion with two endites: first endite with one anterior seta (Fig. 2G: 1) and one posterior seta (Fig. 2G: a); and second endite with one anterior seta (Fig. 2G: 2) and two posterior setae (Fig. 2G: b, c). Gnathobasic filter plate in inner portion, with numerous setae.

Limb V small oval pre-epipodite (Fig. 2H) beside large ovoid epipodite (Fig. 2H: epp). Oval exopodite (Fig. 2H: ext) with one large distal and one small seta nearly half length of large seta. Inner limb portion one setulated ovoid lobe and two unequal plumose setae (Fig. 2H: 1–2).

Ephippial female (Fig. 3A). Morphology as in parthenogenetic female, but with thickened dorsal ridge of valves and gray ephippium containing two resting eggs. Shed ephippium 1.6 as long as wide, with chitinous plate of small polygons. Elongated protuberances with smooth edges in ventral portion and anastomosing lines in posterior portion of ephippium.

Male (Fig. 3B–J). Body ovoid, more elongated than in female; body height/length ratio about 0.46. Dorsal margin of valve slightly higher than cephalic side (Fig. 3B).

Head conical, more elongated than female, lacking fine hairs (Fig. 3B). Dorsal head pore absent, ocellus absent.

Labrum similar to female, thinner (Fig. 3C).

Valve ovoid, more elongated than female (Fig. 3B). Anterior surface lacking fine hairs. Armature of ventral margin of valve similar to that in female. Setae relatively long on anterior portion of ventral margin, shorter on posterior portion (Fig. 3H, I).

Postabdomen similar to that in female (Fig. 3D). Posteroventral angle distinct. Fewer denticles laterally near claw base, with 8–10 feathered setae (Fig. 3D, E). Gonopore open on lateral surface near claw base (Fig. 3D). Small setae on the posteroventral shell rim ungrouped (Fig. 3J).

Antenna I curved, significantly longer than in female, covered by fine hairs and transverse rows of minute denticles (Fig. 3F). Three long antennular sensory setae, implanting at proximal one-third of antenna. Male seta near sensory seta, thicker but shorter. Apex with six short aesthetascs and seven thick hooks.

Limb I long scythe-like appearance, with copulatory hook (Fig. 3G). Exopodite quite long. Stiff seta robust, with blunt tip.

Morphological remarks. The new species can be identified by the diagnostic characters listed below (for an overview, see Table 1). Size relatively large for the genus, with adult parthenogenetic females up to 1.35 mm long. Surface of head and valves without fine hairs. Head without supra-ocular depression and without a dorsal head pore; ocellus absent. On the inner side of the valve, setulae not grouped. Basal pecten on dorsal side of postabdominal claw not prominent; distalmost tooth bident. One additional setule on antenna I. Sensory seta at the basipodite of antenna II long. Anterior stiff setae of limb I

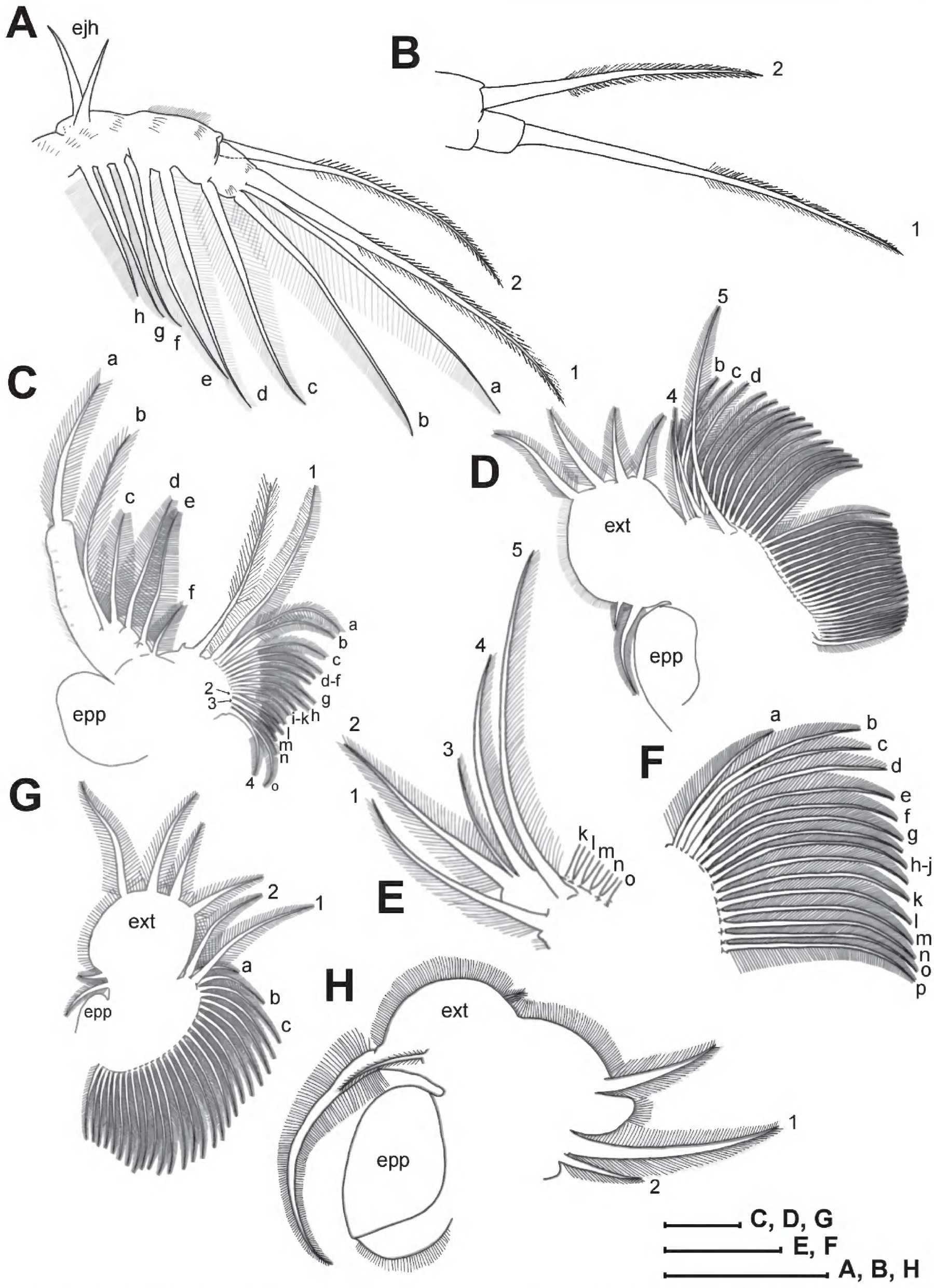

Figure 2. Morphology of *Moina heilongjiangensis*, parthenogenetic females from Xidahai, China. **A.** Limb I; **B.** Stiff setae of limb I; **C.** Limb II; **D.** Limb III; **E.** Inner distal portion of limb III; **F.** Gnathobase of limb III; **G.** Limb IV; **H.** Limb V. Scale bars: 0.1 mm.

bristled. Setae on inner portion of limb II strongly different in size. Setules on stiff setae of limb III and limb IV long. Ephippium containing two resting eggs, its dorsal part with microsculpture represented by small polygons. Male with elongated body. Small setae on the postero-ventral shell rim ungrouped. Antenna I long, three long

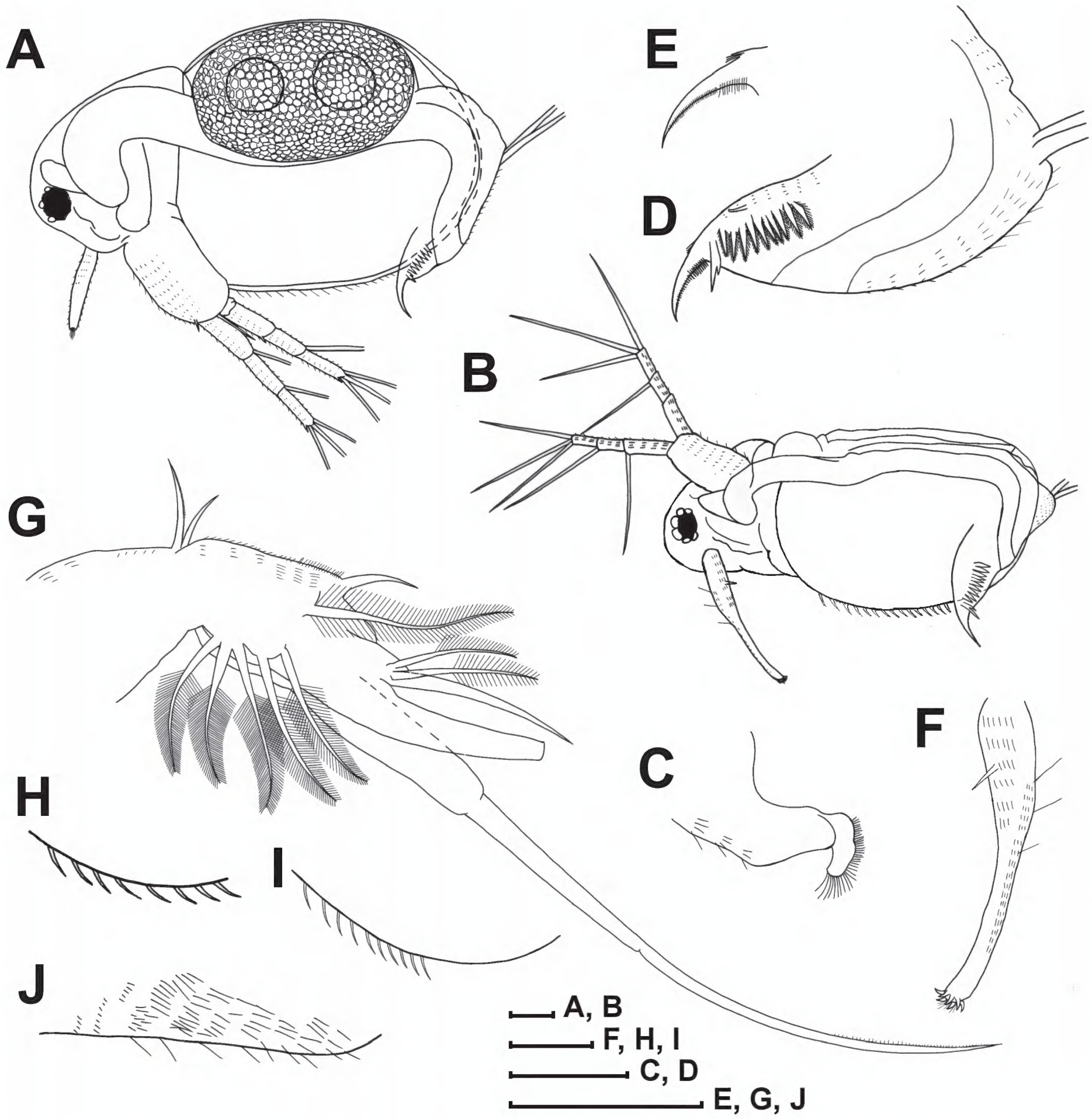

Figure 3. Morphology of *Moina heilongjiangensis*, gamogenetic female (**A**) and male (**B–J**) from Xidahai, China. **A.** Ephippial female, lateral view; **B.** Adult male, lateral view; **C.** Labrum, lateral view; **D.** Postabdomen, lateral view; **E.** Postabdominal claw; **F.** Antenna I; **G.** Limb I; **H.** Anterior portion of valve; **I.** Posterior portion of valve; **J.** Posterior portion of posteroventral shell rim. Scale bars: 0.1 mm.

antennular sensory setae, six short aesthetascs, and seven thick bisegmented hooks of similar size at the apex. Distinctive position of male seta and sensory setae on male antenna I. Thoracic limb I with very long exopodite. Stiff seta of male limb I thick, with blunt tip. The main morphological differences between *M. heilongjiangensis* sp. nov. and *Moina* species with two eggs in the ephippium are listed in Table 1.

Phylogeny. We obtained two unique COI haplotypes and four ITS-1 sequences from two *Moina* individuals from Xidahai (Suppl. material 1: tables S1, S2). In agreement with the morphological examination (Figs 1–3), our COI and ITS-1 Bayesian trees (Figs 4, 5) revealed that the two individuals from Xidahai are distinct from all other *Moina* species in the Bayesian trees, forming an independent clade. This was further supported by three independent species-delimitation methods (i.e., GMYC, bPTP, and ABGD; Fig. 5). In the ITS-1 Bayesian tree, bPTP identified an additional lineage that was not recognized in GMYC and ABGD analyses. Additionally, the same tree showed that *M. heilongjiangensis* sp. nov. is located close to the *M.* cf. *macrocopa* clade, but the COI Bayesian tree suggested a closer relationship to *M.* cf. *affinis* (for which no ITS-1 sequences are available).

Distribution. Known only from the type locality.

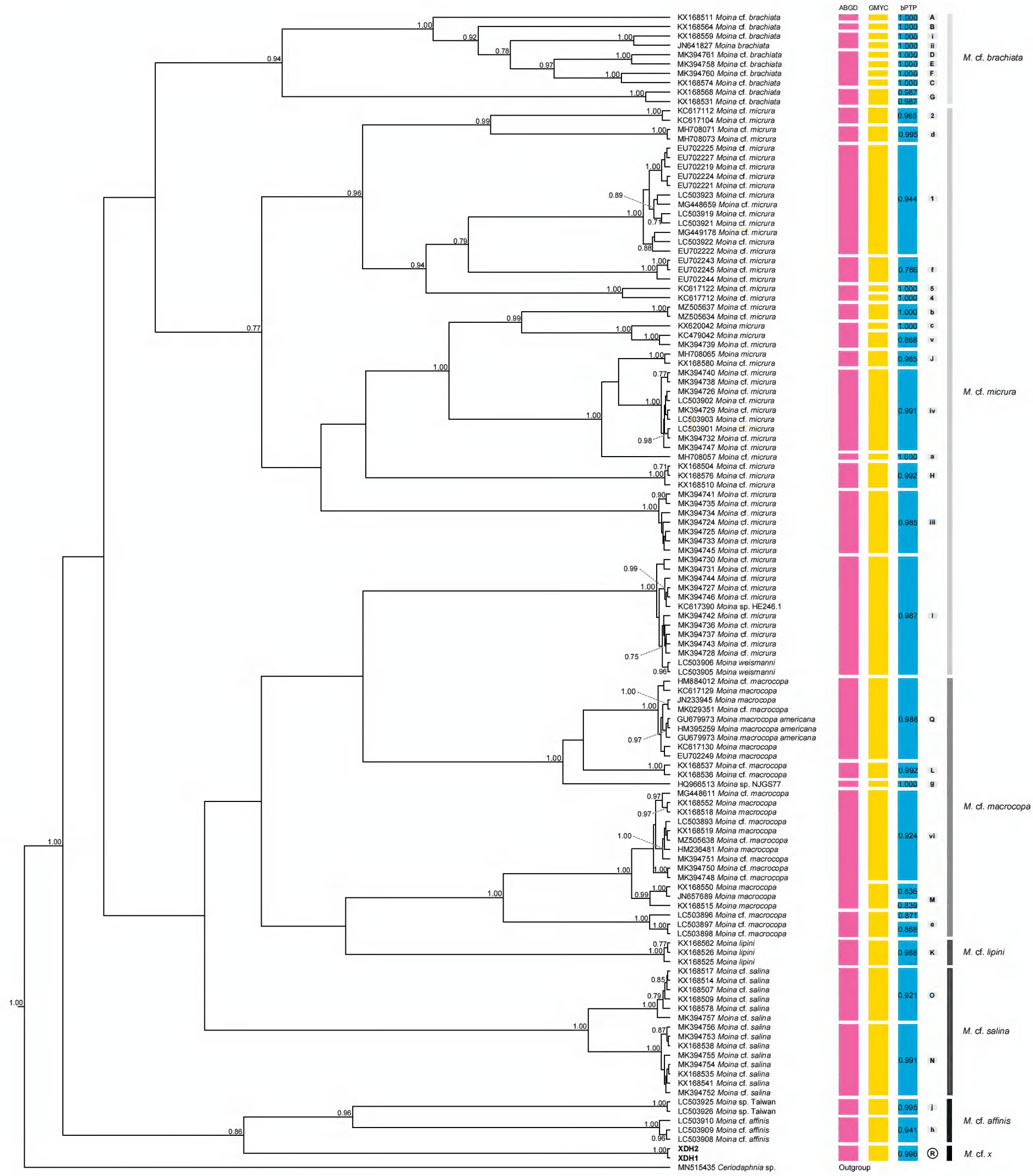

Figure 4. Bayesian phylogenetic tree and lineage delimitation for *Moina*, based on the mitochondrial COI gene (544 bp). Haplotypes of *Moina heilongjiangensis* sp. nov. from Xidahai, China (this study), and previously published sequence IDs are provided in Suppl. material 1: table S1. Posterior probabilities higher than 0.70 are shown. Lineage delimitation according to the ABGD, GMYC, and bPTP methods is indicated, and the lineage ID for *M. heilongjiangensis* is shown in a circle at the right. For the bPTP method, the statistical support for clade membership is also shown. *Ceriodaphnia* was used as an outgroup.

Discussion

Moina species with strongly tuberous ephippia have one or two resting eggs in the ephippium (Neretina et al. 2020). *Moina* species with two resting eggs are larger than those with a single egg. Specifically, parthenogenetic females of *Moina* species with two eggs are longer than 1 mm, whereas parthenogenetic females of *Moina* species with a single egg are equal to or less than 1 mm in length (Kotov et al. 2005; Van Damme and Dumont 2008; Neretina et al. 2020).

We can discriminate *M. heilongjiangensis* sp. nov. from *Moina* species with a single egg in the ephippium:

Table 1. Comparison between *M. heilongjiangensis* and some species of the genus *Moina* Baird, 1850, with two eggs in the ephippium (Goulden 1968; Smirnov 1976; Hudec 1997; Martinez-Jeronimo et al. 1997; Hudec 2010; Montoliu-Elena et al. 2019; Neretina and Kirdyasheva 2019; Neretina et al. 2020; Neretina et al. 2024).

Character	*M. heilongjiangensis* sp.nov.	*M. macrocopa* (Straus, 1820)	*M. ephemeralis* (Hudec, 1997)	*M. hutchinsoni* (Brehm, 1937)	*M. wierzejskii* (Goulden, 1968)	*M. australiensis* (Sars, 1896)	*M. belli* (Gurney, 1904)
Female							
Superficial hairs on the body	Absent	Present	Absent	Absent	Present	Present	Present
Supra-ocular depression	Absent	Absent	Strong	Present	Absent	?	?
Sensory seta at the basipodite of antenna II	Long	Short	?	Relatively long	Long	?	?
Anterior stiff setae (1, 2) of limb I	Bristled	Toothed	Bristled	Bristled	Bristled	Bristled	Bristled
Basal pecten on dorsal side of postabdominal claw	Not prominent	Not prominent	Not prominent	Not prominent	Rather prominent	Not prominent	Not prominent
Distalmost tooth on postabdomen	Bident	Bident	Bident	Single-dent, not plumose	Bident	Bident	Bident
Male							
Number of hooks on antenna I	7	6	8	6	4 (5)	4 (5)	6 (7)
Long exopodite on thoracic limb I	Present	Present	Present	Absent	Absent	Present	Present
Small setae on the posteroventral shell rim	Ungrouped	Ungrouped	Grouped	Grouped	Ungrouped	?	?

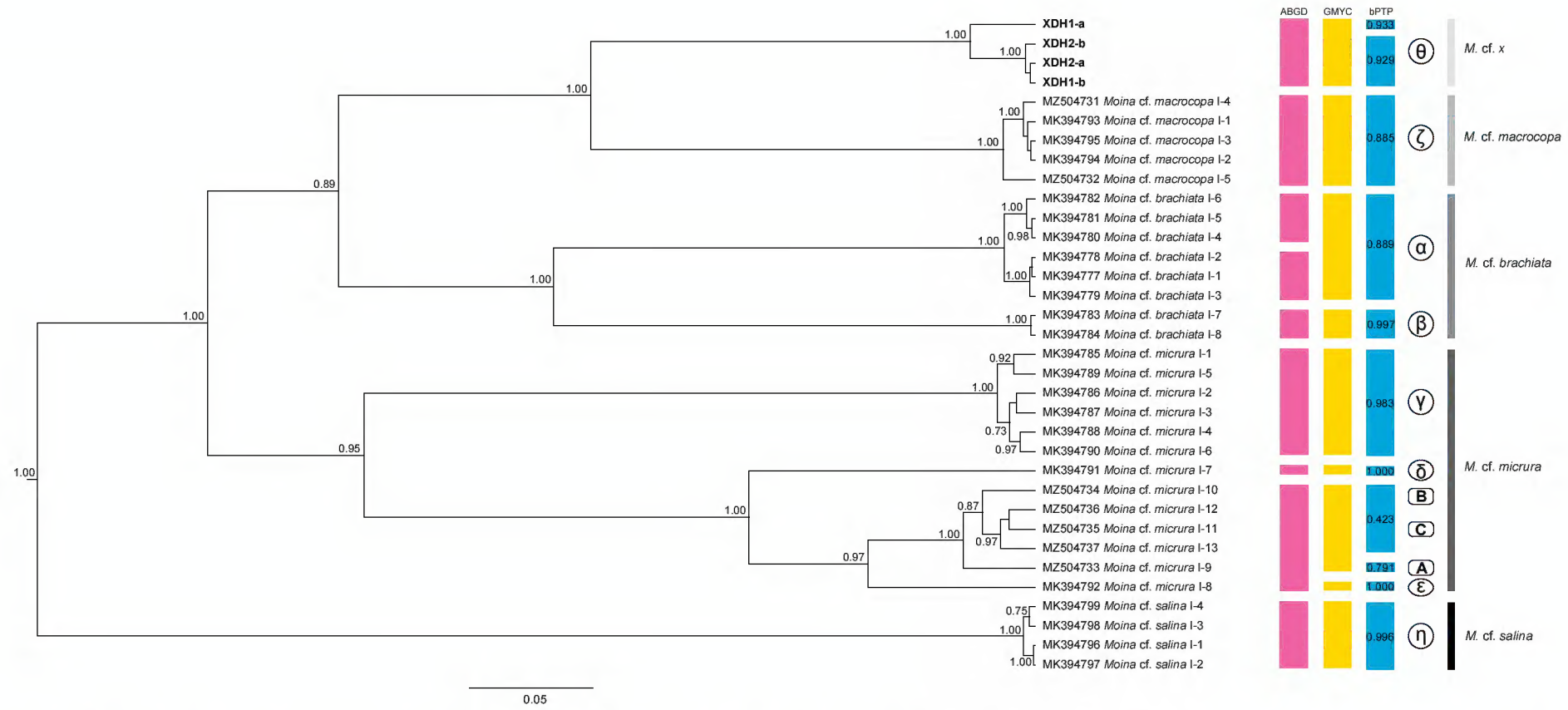

Figure 5. The Bayesian phylogenetic tree inferred from the ITS-1 gene (734 bp) of *Moina*. The taxon names are based on the COI phylogeny. Posterior probabilities higher than 0.70 are shown. Lineage delimitation according to the ABGD, GMYC, and bPTP methods is indicated. For the bPTP method, the statistical support for clade membership is also shown. The list of ITS-1 alleles is provided in Suppl. material 1: table S2.

M. weismanni Ishikawa, 1896 (see description in Hudec 2010), *M. brachiata* (Jurine, 1820) (see description of *M. rectirostris* in Chiang and Du 1979), *M. micrura* Kurz, 1874 (see description in Elías-Gutiérrez et al. 2019), *M. eugeniae* Olivier, 1954 (see description in Smirnov 1976), *M. dumonti* Kotov, 2005 (Kotov et al. 2005), *M. oryzae* Hudec, 1987 (Hudec 1987), *M. mukhamedievi* Mirabdullaev, 1998 (Mirabdullayev 1998), *M. diksamensis* Van Damme & Dumont, 2008 (Van Damme and Dumont 2008), and *M. siamensis* Alonso, 2019 (Alonso et al. 2019).

Among *Moina* species with two eggs in the ephippium (Table 1), we can distinguish *M. heilongjiangensis* sp. nov. from others based on the key morphological characteristics, including the absence of superficial hairs on the body, the presence of the supra-ocular depression, the length of the sensory seta at the basipodite of antenna II, anterior stiff setae of limb I, length of hairs on the preanal portion of the postabdomen, the distalmost tooth on the postabdomen, the number of hooks on the antenna I of the male, the long exopodite on thoracic limb I of the male, and the small setae on the posteroventral shell rim of the male. *Moina heilongjiangensis* sp. nov. and *M. ephemeralis* Hudec, 1997 (see description in Hudec 1997) are the only large moinids with two eggs and covered by superficial hairs on the body, but they differ by the absence of a supra-ocular depression in the new species (well developed in *M. ephemeralis*). *Moina hutchinsoni* Brehm, 1937 (see description in

Martinez-Jeronimo et al. 1997) has a diagnostic morphological characteristic, a single-dent distalmost tooth on the postabdomen, which is different from all other *Moina* species with two eggs. Also, we can distinguish *M. heilongjiangensis* from *M. macrocopa* (Straus, 1820) (see description in Montoliu-Elena et al. 2019) by its relatively long sensory seta at the basipodite of antenna II (short in *M. macrocopa*), bristled anterior stiff setae of limb I (toothed in *M. macrocopa*), and short hairs on the preanal portion of the postabdomen (long in *M. macrocopa*). We can distinguish *M. heilongjiangensis* from *M. wierzejskii*, Goulden, 1968 (see description in Neretina et al. 2020) by its possession of a long exopodite on thoracic limb I of the male (absent in *M. wierzejskii*). *Moina americana* Goulden, 1968, *M. belli* Gurney, 1904, and *M. kaszabi* Forró, 1988, are morphologically close to *M. australiensis* Sars, 1896. These four species have superficial hairs on the body and relatively long hairs on the preanal portion of the postabdomen (Neretina and Kirdyasheva 2019; Neretina et al. 2024), which differ from *M. heilongjiangensis*, which lacks superficial hair on the body and has only short hairs on the preanal portion of the postabdomen.

Among the *Moina* species with two eggs in the ephippium, *Moina macrocopa* is widely distributed in the Palearctic, covering Eurasia, North Africa, and South America (Goulden 1968; Dumont et al. 1981; Korovchinsky 2013; Bekker et al. 2016; Ghaouaci et al. 2018; Montoliu-Elena et al. 2019; Ni et al. 2019; Deng et al. 2021; Neretina et al. 2024). Other species of *Moina* with two eggs in the ephippium have limited geographical distributions. Reliable records suggest that *M. belli* is restricted to some temporary water bodies of the Afrotropics (Rumes et al. 2011; Mengistou 2016). *Moina australiensis* and *M. tenuicornis* are endemic species in Australia (Neretina and Kirdyasheva 2019). *Moina kaszabi* is recorded from Mongolia (Neretina et al. 2024) and Asian Russia bordering Mongolia (Neretina et al. 2024). Records of the *M. lipini* clade are from the central and southern portions of European Russia (i.e., at the border of Kazakhstan) (Bekker et al. 2016). *Moina americana*, *M. hutchinsoni*, and *M. wierzejskii* coexist in North America. *Moina hutchinsoni* and *M. wierzejskii* are restricted to North America, and *M. americana* has confirmed records in Bolivia (Goulden 1968; Martinez-Jeronimo et al. 1997; Martínez-Jerónimo et al. 2004; Montoliu-Elena et al. 2019). *Moina brachycephala* is only recorded in western North America (Goulden 1968), and *M. ephemeralis* only in Central Europe (Hudec 1997). In the present study, *M. heilongjiangensis* sp. nov. is so far recorded in a single brackish lake in Northeast China, but it might have a wider distribution.

Here, we have also constructed molecular phylogenetic trees and used three independent species-delimitation methods (i.e., GMYC, bPTP, and ABGD) to distinguish species and to assess the position of the new species in the genus *Moina*. Our COI tree showed that *M. heilongjiangensis* sp. nov. has a close phylogenetic relationship with *M. affinis*, which is a brackish-water species complex, with a single egg in the ephippium distributed in East Asia, North America, and Europe (Italy) (Yoon and Kim 1992). There are several morphological similarities between *M. affinis* and *M. heilongjiangensis* sp. nov. For example, both species have relatively long setae on the anterior portion of the ventral margin and shorter setae on the posterior portion, a bident tooth near the anterior portion of the postabdominal claw, and an ephippium covered with chitinous plates of small polygons. Additionally, they have one long and thin sensory seta originating from near the bend of male antenna I (Chiang and Du 1979; Yoon and Kim 1992).

Interestingly, we found that our *M. heilongjiangensis* sp. nov. was located close to the *M.* cf. *macrocopa* clade with high support based on the nuclear gene but was close to *M.* cf. *affinis* based on the mitochondrial gene. This may indicate ancient interspecific introgression among *Moina* species, such as has been observed in many taxa of animals (Toews and Brelsford 2012). Mito-nuclear discordances have been frequently observed in Cladocera (e.g., Thielsch et al. 2017; Ni et al. 2019), and hybridization and subsequent introgression might be a key driver of this (Linnen and Farrell 2007; Gompert et al. 2008). However, it is difficult to directly compare the two trees. Our tree inferred from mitochondrial data includes many more sequences and species. Importantly, no ITS-1 sequences of *Moina* cf. *affinis* are available, so this taxon is not represented in Fig. 5. Relevant crossing experiments and/or application of high-resolution nuclear markers are needed to investigate possible introgression/hybridization in the genus *Moina*.

Moina heilongjiangensis sp. nov. is so far known only from the type locality, a brackish lake (salinity: 2.2 ppt) in northeast China. The new species is assumed to be euryhaline, as it can live in a brackish water habitat but cannot survive in freshwater (data not shown). Thus, we assume this species could be present in brackish waters in northern China and southern Siberia. Indeed, its close relatives, *M.* cf. *brachiata*, *M.* cf. *salina*, *M.* cf. *micrura*, and *M.* cf. *macrocopa*, have already been recorded in brackish waters in this region based on morphological examination and phylogenetic studies (Goulden 1968; Chiang and Du 1979; Bekker et al. 2016; Ni et al. 2019; Neretina et al. 2024). We call for further studies to investigate the geographical distribution and the adaptation to brackish waters of this new species.

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Supplementary material 1

Additional information

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Data type: pdf

Explanation note: **table S1.** List of COI sequences of *Moina* used in the COI Bayesian phylogenetic tree. **table S2.** List of ITS-1 sequences of *Moina* used in the ITS-1 Bayesian phylogenetic tree.

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